*DOI:*
10.1038/s41586-018-0073-7

*Document Version*
Peer reviewed version

# Diverse mechanisms for endogenous regeneration and repair in mammalian organs

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## Preface

Mammalian organs comprise an extraordinary diversity of cell and tissue types. Regenerative organs such as the skin and gastrointestinal tract use resident stem cells to maintain tissue function. Organs with less cellular turnover, such as the liver and lungs, largely rely on proliferation of committed progenitor cells. In many organs injury unveils plasticity both of resident stem cells and differentiated cells. The ability of resident cells to maintain and repair organs diminishes with age, yet paradoxically cancer risk increases. New therapeutic approaches aim to harness cell plasticity for tissue repair and regeneration while avoiding the risk of malignant transformation.

## Introduction

The field of cell and gene therapy is now starting to mature, as reports of clinical safety and efficacy in patients has begun to grow[1]. While the number of patients treated is small, autologous human induced pluripotent stem cells (hIPSC) as a treatment for macular degeneration appears to be safe[2], and the ability of gene corrected autologous epidermal cells to provide a long lasting cure for a skin blistering disease has been established[3]. The commercial and academic sectors have also been buoyed by the successes of cancer immunotherapies such as CAR T cell treatments[4].

Cell, tissue and organ replacement is needed to treat irreversible organ failure. Yet in many cases of damage or disease, a better strategy might be to maintain and

reinvigorate organ function in situ. This is an attractive option because it is likely to be less invasive and more cost effective than transplantation. Stimulating endogenous repair by manipulating stem cells and/or their niche is of growing interest, particularly as we come to understand more about the components of the cellular microenvironment[5] and start to exploit the recently revealed plasticity of stem cells in adult tissues[6].

Here we discuss different strategies for tissue repair and regeneration and consider the mechanisms by which cell plasticity is induced in adult tissues. We consider the extent to which induced plasticity might be beneficial in reversing tissue aging and the potential risk of plasticity-associated cancer. Finally we speculate on the feasibility of stimulating endogenous repair and regeneration to treat disease.

**Some definitions**

The stem cell field has evolved to the point where past terminology and dogma are outdated in the context of the adult mammal and so it is worth revisiting some of the terminology. 'Repair' is probably the most straightforward term because it can be defined as restoring damaged tissue to good condition following insults such as ageing, disease and injury. Tissues differ widely in their ability to repair, with the skin and gastrointestinal tract examples of tissues that repair very effectively and the brain a tissue in which repair is highly inefficient. In the case of skin (Fig. 1), while repair efficiently restores the barrier properties of the tissue, the repaired tissue may not be entirely normal; for example there may be scar formation and the repaired skin may be devoid of hair follicles. In situations in which new hair follicles form following wounding[7,8] we can consider them to have regenerated (Fig. 1). We can consider the ongoing replacement of epithelial cells that are shed from the surface of the skin or the lumen of the gut to constitute 'regeneration', essentially a high rate of cell turnover under steady-state conditions. Thus we would distinguish regeneration as not being obligatorily linked to tissue repair.

We now appreciate that while daughter cells that exit the stem cell compartment become increasingly lineage-restricted, they may retain the potential to revert to a stem cell state. This ability of a more differentiated cell to give rise to a stem cell, or the interconversion of distinct stem cell populations, constitutes 'plasticity' and has strong parallels with the terms 'de-differentiation' and 'transdifferentiation' that are well known from classical embryology[9] (Fig. 2). Furthermore, the phenomenon of plasticity suggests that the historical concepts of cell fate specification and

determination are not, as previously thought, irreversible. Plasticity also calls into question the meaning of 'terminal differentiation', which is classically used to describe a unidirectional process by which post-mitotic differentiated cells are formed from a stem cell population. When such a cell lacks a nucleus, as in the case of human red blood cells or cells in the outer layers of human epidermis, there is no doubt that the state of differentiation is 'terminal', as in 'irreversible'. However, there are now many examples across organ systems where cells committed to a specific lineage can re-enter the stem cell compartment following injury[6,10]. Therefore it would appear that even if the trajectory of a cell is towards a terminal state, different steps along the route may be reversible.

Finally, what do we mean by 'quiescence'? Historically, this refers to cells that are not actively dividing and may have arrested in G0 phase of the cell cycle. If such cells incorporate a DNA label in S phase they may retain the label over many months or even years; however, these DNA label retaining cells are not obligatorily arrested in G0[11]. In stem cell biology, quiescence is now generally taken to mean a dormant genome (heterochromatinisation and low levels of global transcription), and metabolic dormancy (low protein translation and lack of oxidative phosphorylation)[12,13].

**Cell lineage tracing: the caveats**

Inevitably, much of our understanding of the nature and properties of adult tissue stem cells comes from lineage tracing studies in mice. This is powerful technology, allowing us not only to trace the progeny of individual cells over many generations, but also to selectively ablate or expand specific populations. However, there are some technical contstraints that must not be ignored. One is so elementary that it should not need to be discussed: it is unwise to assume that a marker that is expressed in a particular stem cell population is not also expressed in other cells within the tissue. A good example here is Lrig1, which is an excellent epithelial stem cell marker in a range of tissues, including the epidermis, yet is also expressed in a highly dynamic fashion in a subset of skin fibroblasts[14]. Except in pathological situations, where potentially epithelial cells invade the underlying connective tissue, or cells undergo epithelial-mesenchymal transition, the gene of interest can still provide a highly useful reporter. Indeed Lgr5 and Lgr6, widely used as stem cell markers in a range of epithelia, are also expressed by mesenchymal populations in the lung[15].

Co-expression of the stem cell marker Lgr5 in differentiated cells or de novo expression following injury may have led to some confusion as to the source of regenerating cells. Following injury, increased numbers of Lgr5-lineage traced cells have been seen in proliferative organs such as stomach[16] and skin, as well as relatively slow cycling organs like the liver[17] and pancreas[18]. In the stomach Lgr5 is expressed in stem cells as well as chief cells following injury, and this highlights an ongoing debate on whether chief cells contribute to the regenerating gastric epithelim (Text Box 1).

The use of dual recombinase systems can overcome this weakness since lineage tracing relies on co-expression of two factors in the same cell[19]. This approach was used to revisit the concept that biliary epithelial cells can transdifferentiate into hepatocytes following injury, a concept for which there are opposite results (Text Box 1). By using two separately encoded recombinase proteins (Sox9-CreER and Alb-DreER) they demonstrated that Sox9-expressing biliary epithelial cells did not form hepatocytes after injury. This is consistent with the emerging concept that biliary epithelial cells give rise to hepatocytes only occurs under conditions when hepatocytes are unable to divide[20,21].

Other approaches for lineage tracing include barcoding and exploitation of naturally occurring DNA mutations. For example, Pei et al[22] have recently developed an artificial DNA recombination locus (termed Polylox) for barcoding based on Cre-loxP recombination. Polylox recombination in situ generates several hundred thousand barcodes, allowing tagging of single cells in mice. Pei et al used this for fate mapping of haematopoietic stem cells in mice, allowing them to demonstrate that the adult haemopoietic stem cell compartment is a mosaic of clones that were present in the embryo and that most clones in the adult have multilineage differentiation potential. Newly developed CRISPR/Cas-based systems to generate large-scale maps of cell lineage in multicellular systems for normal development and disease[23].

In terms of human lineage tracing, sun-exposed human skin has been a tractable system for many years and recent studies have inferred the clonal architecture of the epidermis based on DNA sequencing[24,25]. In this case, a caveat that has emerged is that the interpretation of the data depends on how much skin is sequenced to capture the largest mutant clones. When sufficiently large areas of skin are sequenced, it is possible to infer the effects of primary and secondary mutations, leading to the conclusion that secondary mutations arising at the edge of a mutant

clone have a selective growth advantage[26]. Large scale single cell gene expression profiling of adult human tissues will undoubtedly improved our understanding of the inter-relationships between cell states, cell types and lineages[27].

**Cell intrinsic mechanisms of plasticity**

Plasticity of adult tissues can be manifest as dedifferentiation or transdifferentiation from one stem cell type into another[28] (Fig. 2). While in most cases plasticity is linked to injury, this is not an obligatory association. In the epidermis, for example, switching between stem cell populations and fate switching between differentiated lineages can be induced by β-catenin activation in undamaged epidermis[29]. It is possible that this occurs because the epidermis in which β-catenin is activated can reprogram the underlying connective tissue, the dermis, to a neonatal state via secretion of signalling molecules such as Sonic hedgehog[8,30]. In support of this, the hair follicle defects resulting from deleting the transcriptional co-activator Blimp1 in the dermis are rescued by epidermal β-catenin activation[31].

There are also many examples of exploiting transcription factors to induce plasticity by modulating gene expression cell autonomously. This is evident in the pancreas, which exhibits very limited regenerative potential under homeostatic conditions yet in which a latent multipotent cell type can be uncovered within the adult pancreas[32]. Expression of endocrine-promoting transcription factors in the exocrine cells of the pancreas triggers their conversion to insulin-expressing cells capable to reversing diabetes[33]. Inactivation of the SCF-type E3 ubiquitin ligase substrate recognition component Fbw7 induces pancreatic ductal cells to reprogram into α, δ, and β cells and acts by stabilising the transcription factor Ngn3, a known regulator of endocrine cell differentiation[34].

**Injury-induced plasticity**

The induction of pluripotency by the expression of transcription factors demonstrates that plasticity can be experimentally induced in virtually all somatic cells. However in nature, plasticity is most commonly associated with tissue injury and damage (Text Box 1). Injury can be manifest in a number of ways, such as targeted laser ablation[35], tissue disaggregation[10] or full-thickness wounds[7,36] in the skin. In the intestine, DTR-mediated stem cell ablation[37] or 5-fluoro-uracil mediated killing of proliferative cells[38] causes secretory and absorptive progenitors to acquire ISC-like properties and contribute to regeneration. A rare population of cells in the crypt that appear to be

enteroendocrine cells also express ISC markers and can broadly contribute to epithelial regeneration following irradiation[39]. While DTR-mediated killing of genetically marked cells is not a normal physiological condition, the effects of *Helicobacter pylori* in the stomach undoubtedly are. Chronic injury caused by *Helicobacter pylori* infection can directly amplify the regenerative response and cause a number of epithelial changes including loss of Parietal cells and confusion of cellular identity of chief cells[40].

Plasticity is not limited to the epithelial compartment of tissues. For example, Plikus et al[41] have reported regeneration of adipocytes from dermal myofibroblasts during skin wound healing. This occurs through interaction with hair follicles that are forming in the wound. The hair follicles trigger bone morphogenetic protein (BMP) signaling and activation of adipocyte transcription factors. Indeed it has been suggested that fibrosis may not be solely a consequence of aberrant signalling but by aberrant differentiation of a particular subpopulation of fibroblasts[42].

**Inflammation and infection induced plasticity**

Microbe- and injury-induced inflammatory responses are mediated by cells of the innate immune system, which produce and respond to cytokine mediators and other inflammatory signals. Tissue resident cells sense these signals and respond by migrating, proliferating and regenerating the tissue[43]. Many of the mechanisms that link inflammation to damage repair and regeneration in mammals are conserved in lower organisms, indicating that it is an evolutionarily important process[44]. In terms of immune modulation of stem cells and regeneration, evolutionary advances in the immune system appear to be inversely correlated with the ability to fully regenerate injured tissues[45].

There are a number of ways in which the immune system can facilitate endogenous repair. For example, there is a strong link between macrophage-mediated debris clearance and regeneration in the central nervous system (CNS)[45] and macrophages are essential for effective skin wound healing[46]. In addition, immune cells can activate proliferation: in undamaged skin Notch-mediated communication between regulatory T cells (Tregs) and hair follicle stem cells is required for hair follicle growth[47], while Tregs activate muscle satellite cells following injury[45]. In addition, TIE2-expressing monocytes/macrophages promote revascularization of ischemic limbs[48].

Another way inflammation facilitates repair is through modulating stem cell regulatory proteins like Lgr5. Lgr5 is a Wnt target gene and macrophages are a known source of Wnt ligands in a variety of contexts including ulcerative colitis[49]. Lgr5 is also directly regulated by inflammatory mediators. In the intestine, the NfKβ signaling effector Stat5 regulates the increase in Lgr5-expressing intestinal stem cells in response to injury. In basal cell carcinoma, Stat3 directly binds to and regulates transcription of Lgr5[50]. In the intestine Interleukin-22 acts via Stat3 to promote epithelial regeneration[51]. Therefore immune cells could mediate an increase in plasticity through direct regulation of stem cell pathways.

In addition to these roles for inflammation in tissue repair, recent data suggest that inflammation can trigger epigenetic memory of injury[52] and that innate immune signalling can play a role in nuclear reprogramming ('transflammation'[53]), for example via reactive oxygen species (ROS). If inflamed skin is wounded, heals and is then re-wounded, the second wound heals more rapidly[52]. This is because epidermal stem cells maintain chromosomal accessibility at key stress response genes that are activated by the original inflammatory stimulus. This memory depends on the intracellular DNA sensor absent in melanoma 2 (*Aim2),* which encodes an activator of the inflammasome. In the absence of AIM2 or its downstream effectors the epidermal memory is erased. More broadly, intracellular nucleic acid sensors can discriminate between self- and non-self-nucleic acids[54], opening up the possibility that pathogens can induce plasticity in adult tissues by a similar mechanism.

**Cell-ECM and cell-cell adhesion**

In addition to inducing inflammation, injury induces changes in the adhesive interactions between cells and their environment. Epithelial damage can lead to loss of basement membrane proteins and deposition of new extracellular matrix with a different protein composition. It can also lead to loss of neighbouring cells and interactions with new neighbours, linked, for example, to cell migration[10]. Inevitably, the fine patterning of niches, leading to local and spatially distinct signals in undamaged tissue[55], is destroyed following tissue damage.

Recent studies demonstrate that physical factors play a key role in determining tissue architecture under steady state conditions. For example, the undulations of the human epidermal-dermal junction are instructive in epidermal stem cell clustering[56]. Crowding in the epidermal basal layer affects cell shape; a decrease in cortical tension and increased cell-cell adhesion trigger differentiation and movement of cells

into the suprabasal layers[57]. Piezo1 plays a role in linking mechanical stretch to division of epithelial cells[58]. One of the key integrators of cell position and cell fate in the epidermis and other tissues is YAP activity[59,60,61]. YAP integrates diverse signals, including Notch, Wnt and ECM signals[61,62] and intercellular adhesion[60]. In the mouse YAP plays a key role in cell fate transitions during colonic regeneration following injury[62]. The colonic epithelium is transiently reprogrammed into a primitive, fetal-like state that is orchestrated by ECM remodelling, FAK/Src signalling and YAP activation. The combination of ECM proteins and Wnt ligands is sufficient to sustain endogenous YAP/TAZ and induce conversion of cell fate.

One way in which injury can potentially induce plasticity is via modulation of the actin cytoskeleton. For example, Rho kinase inhibition expands epithelial stem cells in culture, including cells from mammary gland, prostate, intestine and colon[63,64]. Similarly, inhibition of dual SMAD signalling enables long-term expansion of basal cells in a variety of epithelia, both in vivo and in vitro[65]. TGFβ/BMP/SMAD signalling is activated in the differentiated cells of multiple epithelia, including lung, mammary gland, stomach and epidermis, and is suppressed in basal cells that express the transcription factor p63. In lung airway epithelium, SMAD signalling promotes differentiation, while SMAD inhibition leads to stem cell hyperplasia. These findings are likely to be relevant in epithelial cancers. For example, suprabasal expression of the $\alpha$6β4 integrin in mouse epidermis increases tumour formation in part by relieving TGFβ-mediated growth inhibition[66].

**Aging and regeneration**

Tissue integrity, organ function and regenerative capacity all decline with age[67]. Despite an obvious relevance to human health, there are relatively few studies about changes in regeneration/homeostasis with age, possibly due to the technical and financial burdens of studying “old” animals. Therefore the causes of this decline are not well understood. Likely factors involved include loss of stem cell number and proliferative capacity, ECM remodeling towards a more fibrotic phenotype, and cumulative DNA damage causing cellular senescence (Fig. 3). It is clear that stem cell extrinsic factors such as fibrosis caused by chronic inflammation hasten these processes. However there are also cell-intrinsic changes in stem and progenitor cells that appear to be largely age associated. Age-related changes in tissue resident stem cells have been observed in a range of tissues, including blood, skin and intestine[67].

Some age related changes are cell-intrinsic since stem cells isolated from aged patients grow poorly in culture relative to their young counterparts. The cell-intrinsic mechanisms driving stem cell aging include loss of telomeres, epigenetic changes[68], decline in mitochondrial function[69] and accumulation of DNA mutations[70]. In the intestine it has been shown that critical telomere shortening inactivates Wnt, which results in stem cell failure[71]. The cellular safeguards to cancer, including programmed cell death, senescence and differentiation, might be responsible for reduced stem cell number and activity as we age. Tomasetti and Vogelstein[70] have discussed how mutations are coupled to the number of stem cell replications and this might cause the well-known increase in cancer risk with age. An increase in mutation load in stem cells would be expected to correlate with increased cell death and senescence, resulting in the loss of stem cell number or proliferative capacity.

The therapeutic possibility of rejuvenating aged organs is best exemplified from parabiosis experiments. Parabiosis, the surgical linking of the circulatory systems between old and young animals, resulted in the restoration of stem cell activity in the organs of old mice. Moreover, a single transfusion of old blood causes a rapid loss of stem cell activity in young mice, demonstrating the negative effects circulating factors in old animals[72,73]. The effects of parabiosis might directly act on stem cells[74] or might act to remodel and rejuvenate the niche[75]. Circulating factors that might modulate the niche include extracellular matrix (ECM) proteins, growth factors, or immune cells. In the skin, age-associated proteolysis of type XVII collagen results in hair follicle minituarisation and epidermal hypertrophy, which can be reversed by reexpressing the collagen[76,77]. Soluble growth factors and cytokines, like Wnt and IL22, can reverse age related decline of intestinal stem cells[51,78]**.** One of the most recent approaches to rejuvenate organs is through the targeted removal of senescent cells[79].

**Plasticity and cancer**

The obvious downside of inducing plasticity for tissue rejuvenation is the risk of cancer. Thus inflammation-induced lineage infidelity in the epidermis is transient in wounds but persists in cancer[36]. Specific chromatin states prime cells in squamous cell carcinoma to undergo EMT[80]. A further finding is that neighbouring wild type cells have the ability to correct aberrant epidermal behaviour of cells harboring β-catenin mutations[81].

There are multiple examples linking cellular plasticity and cancer in the GI tract. In the stomach, induction of plasticity in differentiated cells occurs during chronic injury, and in response to acute injury, where chief cells co-express mucous neck cell markers (TTF2 aka spasmolytic polypeptide), a precancerous lesion termed SPEM (spasmolytic polypeptide expressing metaplasia)[82]. A further example of cancer-associated plasticity is intestinal metaplasia, where esophagus or stomach epithelium converts to an intestinal epithelium. In the case of Barrett's oesophagus, an intestine-like metaplasia and precursor of oesophageal adenocarcinoma is triggered by gastro-oesophageal reflux. Recently it has been suggested that transitional basal cells at the squamous-columnar junction generate Barrett's oesophagus[83].

**Therapeutic prospects**

Treatments that target the niche are already being evaluated to treat cancer[5,84] and this review has highlighted a number of strategies that could be exploited to promote tissue regeneration. Looking into the future, computational analysis of cell state transitions indicates that it may be possible to stabilise particular cell states – whether stem cell, committed cell or differentiated cell – pharmacologically[85] (for therapeutic benefit. In addition, mining large datasets of single cell gene expression profiles[86] will likely enhance our understanding of different cell types and states and the pathways by which they are specified.

One concern is whether the induction of plasticity will ever be therapeutically relevant, given that the proportion of cells in a tissue that exhibit plasticity is often exceptionally rare[37,38]. Time will tell, but it is worth concluding with the observation that gastric bypass surgery in humans enhances pancreatic β-cell function and may also increase β-cell mass[87]. Is this an example of the benefits of injury-induced plasticity?

**Acknowledgements**

FMW gratefully acknowledges funding from the Wellcome Trust (206439/Z/17/Z), Medical Research Council (MR/PO18823/1), Cancer Research UK (C219/A23522) and the Biotechnology and Biological Sciences Research Council (BB/M007219/1). JMW is supported by grants from the National Institutes of Health R01DK092456, U19AI116491, P01HD093363 and U01DK103117.

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**Figure legends**

**Figure 1: Epidermal plasticity (A)** Location of Lgr5, Lgr6 and Lrig1+ stem cells in undamaged adult mouse dorsal epidermis. (**B, C**) Following wounding the interfollicular epidermis may be regenerated in the absence (**B**) of hair follicles and sebaceous glands (epidermal repair) or, alternatively, the interfollicular epidermis and adnexal structures re-form, constituting epidermal regeneration (**C**). **(D)** Following wounding the differentiated cells of the Gata6 lineage that are normally confined to the suprabasal layers of the sebaceous duct are able to enter the wound site, undergo dedifferentiation and contribute to the stem cell compartment of the interfollicular epidermis.

**Figure 2: General mechanisms of plasticity (A)** Under normal homeostatic conditions stem cells self-renew and generate differentiated lineages in a unidirectional manner via committed progenitors. The fate of stem cells – self-renewal or generation of differentiated cells is influenced by signals from the niche, including specific cell types, soluble signals and ECM. **(B)** In the context of tissue injury or disease, stem and progenitor cells and their differentiated progeny exhibit plasticity, including dedifferentiation and transdifferentiation. This is driven, in part, by

altered niche signals, including new interactions with cells of the immune system and ECM remodelling.

**Figure 3: General mechanisms of aging** The balance between stem cell renewal and differentiation and the interactions between stem cells and the niche (A) are altered with aging **(B).** Age-associated changes are both cell intrinsic, such as accumulation of mutations, and niche-associated, such as fibrotic ECM.

**Text Box 1: Examples of plasticity**

**Lung**[28] There are two major compartments in adult lung: the airways and the alveoli. In the airway epithelia, differentiated club cells (secretory cells) can directly convert into ciliated cells. Basal cells replenish all types of secretory and ciliated cells following tissue damage. Club cells can de-differentiate into basal stem cells following stem cell ablation. The alveolar epithelium consists of type 1 cells that permit gas exchange and surfactant-producing type 2 cells. Type 2 cells generate type 1 and type 2 cells under homeostatic conditions and after injury. In addition, after injury type 1 cells can generate type 2 stem cells.

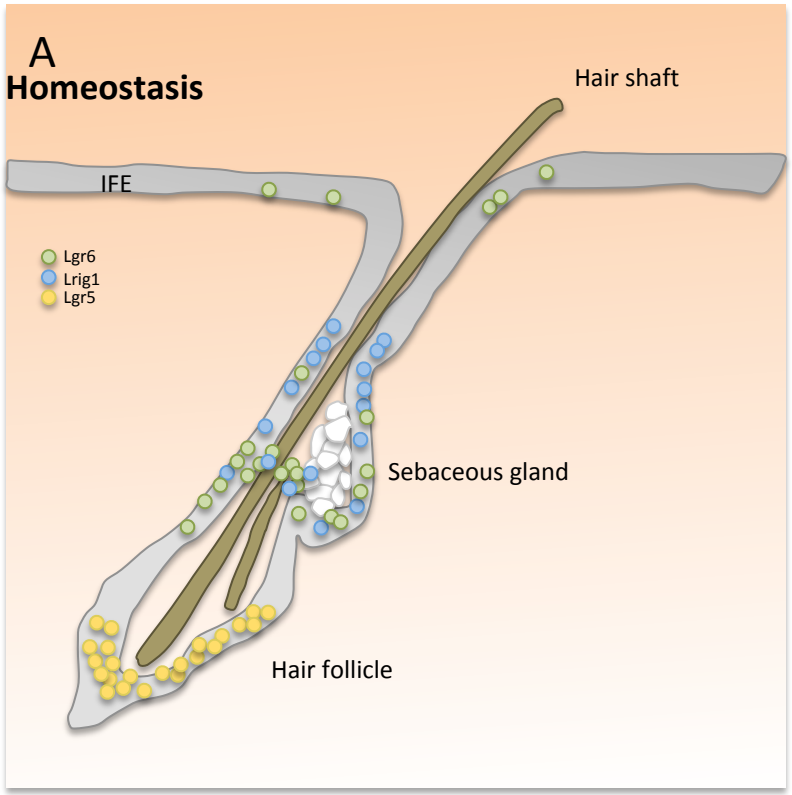
**Stomach** The stomach is regionalized into the proximal corpus and distal antrum (pylorus). The corpus epithelium contains differentiated chief and parietal cells, while the antrum contains gastrin producing G cells. Antrum stem cells express Lgr5[88,89], whereas corpus stem cells express Sox2, Lrig1, Mist1[90,91,92]. Chief cells are long-lived, highly specialized, pepsinogen-producing cells and become recruited to contribute to epithelial repair following injury[16,38].

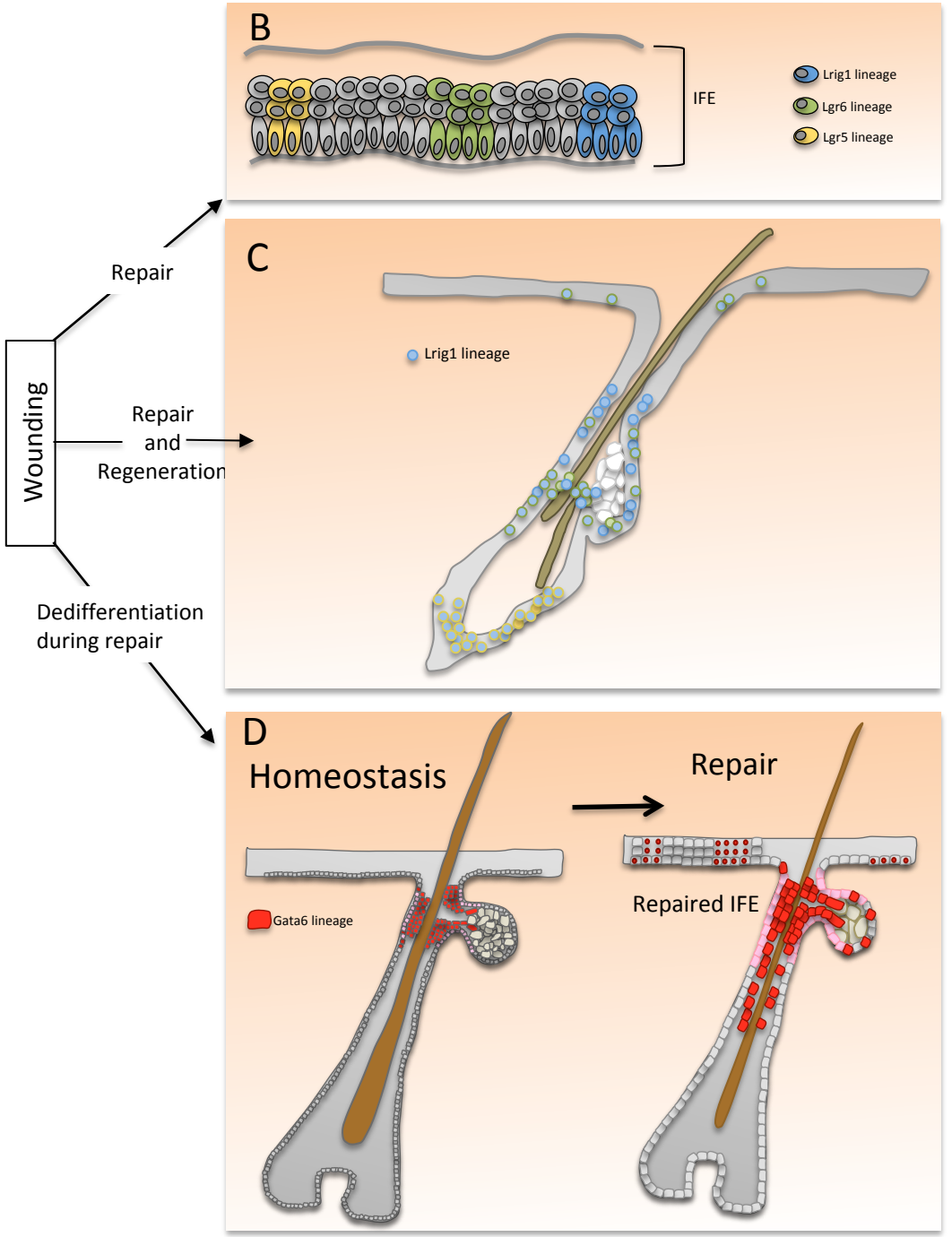
**Liver** The liver is comprised of two cell types, hepatocytes and cholangiocytes. A subpopulation of Wnt-responsive hepatocytes maintain the liver under steady state conditions[93]. After a partial hepatectomy, the remaining hepatocytes divide until the original liver mass is restored and then return to a non-proliferative state[94]. In contrast, when there is acute or chronic damage to hepatocytes such that they are unable to proliferate, cholangiocyte-like cells near the bile duct tree regenerate hepatocytes and cholangiocytes[20,21].

**Similarities in regeneration and plasticity across GI organs** There are vast structural and functional differences in the epithelium of GI organs, ranging from a stratified squamous epithelium with little cellular diversity in the oesophagus, to a complex glandular mucosa in the stomach, to a columnar epithelium in the intestine

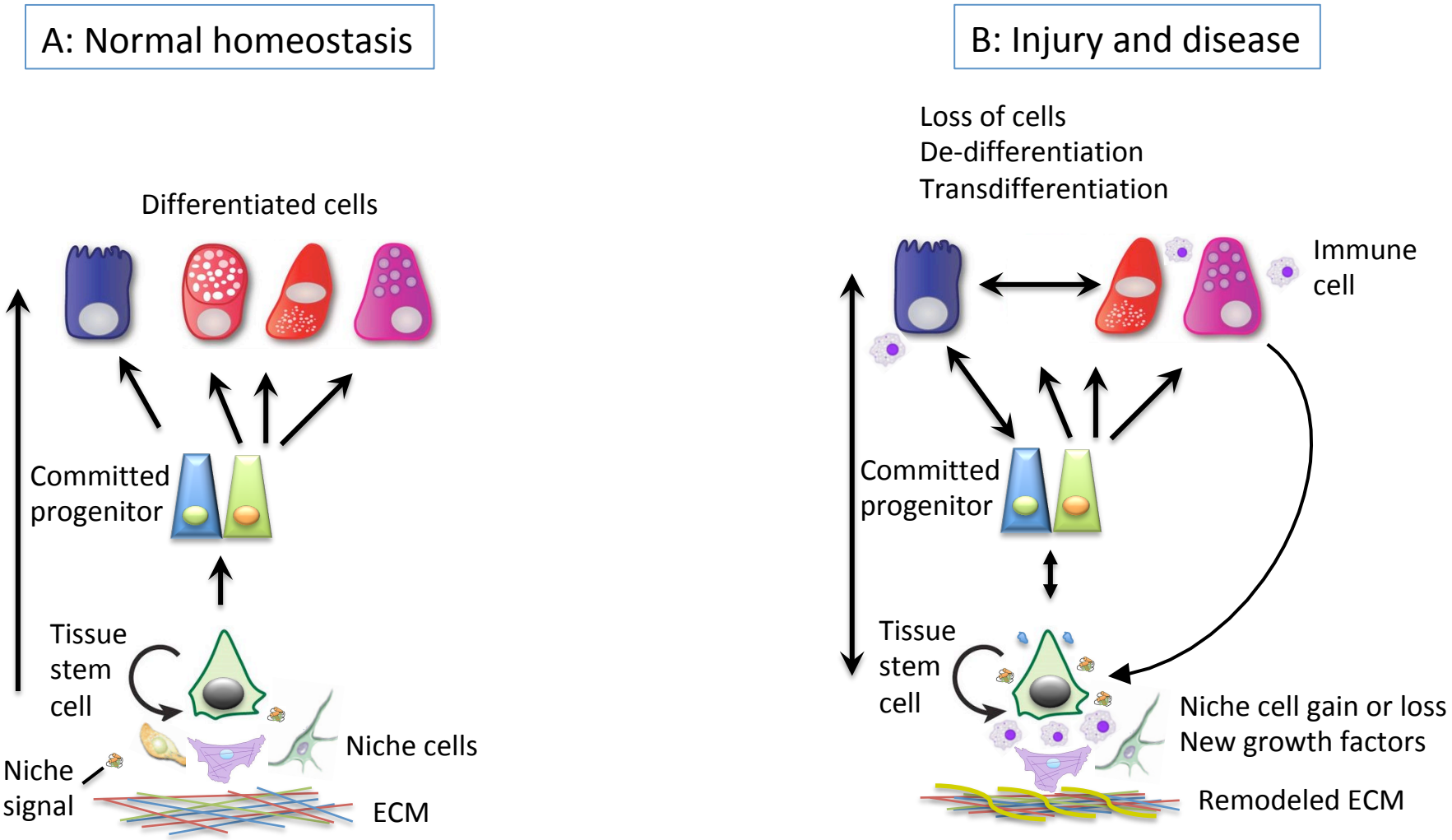
containing a diverse mix of absorptive and secretory cell types. Nevertheless, these organs share many features. The entire epithelium of the GI tract derives from a single layer of embryonic endoderm[95] and similar signaling pathways control epithelial homeostasis in the different organs. Canonical Wnt signaling is involved in maintaining and expanding stem and progenitor cells, whereas BMP and Notch signaling promote differentiation of the various epithelial lineages. Indeed there is evidence that the Caudal Homeobox transcription factor Cdx2 is the single factor that functionally distinguishes an intestinal from a gastric stem cell [96,97]. It is not clear why GI stem cells have so many similarities, but it could be a primitive epigenetic memory due to their shared cellular origin during embryonic development.

# Fig. 1: Epidermal plasticity



# Fig. 2 General mechanisms of plasticity



# Fig. 3: General mechanisms of aging